CONTROL OF COMMON WATERHEMP WITH *S*-METOLACHLOR PLUS FOMESAFEN AND COMPETITIVENESS OF PROTOX-RESISTANT COMMON WATERHEMP

by

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Abstract

Field experiments were conducted near Manhattan, KS in 2005 and 2006 and Sabetha, KS in 2005 to determine the efficacy of *S*-metolachlor tank mixed with fomesafen on common waterhemp in soybean. Preemergence treatments included *S*-metolachlor + fomesafen at 0.91 + 0.22, 1.21 + 0.28, 1.52 + 0.36, and 1.82 + 0.43 kg ha^{-1} and *S*-metolachlor + metribuzin at 0.55 + 0.14 kg ha^{-1}. These treatments were applied alone or followed by a postemergence glyphosate application at 0.88 kg ha^{-1}. Ratings were taken 2, 4 and 8 weeks after treatment. The study showed that *S*-metolachlor + fomesafen gave excellent early season control of common waterhemp at both Sabetha and Manhattan. *S*-metolachlor + fomesafen at the 1.52+0.36 kg ha^{-1} rate gave greater weed control than *S*-metolachlor + metribuzin.

A separate study was conducted to determine the competitiveness and fitness of a protox-resistant common waterhemp biotype. Protox-resistant and protox-susceptible biotypes of common waterhemp were grown under noncompetitive and competitive arrangements in the greenhouse. In the noncompetitive study a single plant of both biotypes was planted in 15-cm-diam pots. Photosynthesis, leaf area, and plant biomass were measured 10, 20, 30, and 40 day after transplanting (DATP). In general, photosynthesis rate and plant biomass was similar between biotypes. However, the protox-resistant biotype had higher leaf area then the susceptible biotype at 20, 30, and 40 DATP.

Under competitive conditions, a replacement series study, photosynthesis, leaf area, plant height, and plant biomass were measured 7, 14, 21, and 28 DATP. In general protox-resistant and –susceptible common waterhemp values were similar 28 DATP.

Relative crowding coefficient values 28 DATP were 0.86, 0.89, 1.09, and 1.13 for photosynthesis, leaf area, plant height, and plant biomass, respectively. Suggesting, protox resistance did not change the ability of common waterhemp to grow normally under competitive conditions.

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CHAPTER 1 - Common Waterhemp Control in Soybean with *S*-Metolachlor Plus Fomesafen or Metribuzin

ABSTRACT

Field experiments were conducted near Manhattan, KS in 2005 and 2006 and Sabetha, KS in 2005 to determine the efficacy of *S*-metolachlor tank mixed with fomesafen on common waterhemp (*Amaranthus rudis*) in soybean (*Glycine max*). Preemergence treatments included *S*-metolachlor + fomesafen at 0.91 + 0.22, 1.21 + 0.28, 1.52 + 0.36, and 1.82 + 0.43 kg ha^{-1} and *S*-metolachlor + metribuzin at 0.55 + 0.14 kg ha^{-1}. These treatments were applied alone or followed by a postemergence glyphosate application at 0.88 kg ha^{-1}. Common waterhemp control with *S*-metolachlor + fomesafen was greater than 88 and 60% at 2 and 8 WAT, respectively at Manhattan in 2005. However, *S*-metolachlor + fomesafen, regardless of the rate, gave complete common waterhemp control 2 WAT and greater than 95% common waterhemp control by 8 WAT at Sabetha. In 2005, *S*-metolachlor + metribuzin controlled 59 and 91% of common waterhemp 8 WAT at Manhattan and Sabetha, respectively. By 8 WAT, glyphosate applied after preemergence improved common waterhemp control; however, no additional control was achieved with the postemergence glyphosate applications at Manhattan in 2006 and Sabetha. Early season control of common waterhemp can be achieved with *S*-metolachlor + fomesafen at 1.52 + 0.36 kg ha^{-1} with or without a postemergence application of glyphosate.

Key words: Herbicide resistance, Protox-inhibitor herbicides, protox resistance.

INTRODUCTION

Common waterhemp (*Amaranthus rudis*) is a troublesome weed throughout the Midwestern United States because of its prolific seed production and rapid growth characteristics (Battles et al. 1998; Bensch et al. 2003). A concern for producers is the ability of common waterhemp to have detrimental effects on crop yield. Research has indicated that common waterhemp densities of 2, 6, and 11 plants per 10 meters of crop row resulted in soybean yield reductions of 5, 10, and 15%, respectively (Bauer et al. 1991). In addition, common waterhemp allowed to compete 10 week after soybean unifoliate leaf expansion before removal reduced soybean yield by 43% (Hager et al. 2002).

Control of common waterhemp in conventional soybean has been achieved with both preemergence and postemergence herbicide applications. Common waterhemp control in conventional soybean has been attained with herbicides that belong to different classes; including dinitroanilines, chloroacetamides, protoporphyrinogen oxidase (protox)-inhibitors, acetolactate synthase (ALS)-inhibitors, and metribuzin (Mayo et al. 1995; Regehr et al. 2007; Sweat et al. 1998). However, common waterhemp populations have developed resistance to herbicides. Heap (2006) reported that common waterhemp with resistance to four modes of action have been identified in the United States including ALS-inhibting, glycines, photosystem II-inhibitors, and protox-inhibiting herbicides.

Common waterhemp with resistance to ALS-inhibiting herbicides was first reported in northeast Kansas in 1993 and several other reports of resistance also have been confirmed throughout the Midwestern United States (Horak and Peterson 1995; Peterson 1999). The first case of common waterhemp with resistance to photosystem II-inhibiting herbicides was reported in southern Nebraska in 1990 (Tranel and Patzoldt 2002). In addition, Foes et al. (1998) reported that a biotype of common waterhemp with multiple resistances to ALS and photosystem II-inhibitors was discovered in Illinois.

Common waterhemp resistance to postemergence applications of protox-inhibiting herbicides was confirmed in northeast Kansas in 2002 (Shoup et al. 2003). This biotype was 8 times more resistant to postemergence applications of fomesafen than a susceptible biotype (Shoup et al. 2003). Common waterhemp biotypes from Missouri and Illinois also have been reported to have resistance to protox-inhibiting herbicides (Li et al. 2004; Patzoldt et al. 2002). While this biotype of common waterhemp is resistant to postemergence application of protox-inhibiting herbicides, Falk et al. (2006) found that preemergence applications of fomesafen gave near complete control of the protox-resistant common waterhemp biotype.

In 2005, the first glyphosate–resistant common waterhemp was confirmed in northwest Missouri (Heap 2006). Glyphosate is widely used to control common waterhemp in soybean with 80% of U.S. soybean treated with glyphosate (Duke 2005). Management of glyphosate resistant weeds may require the use of preemergence herbicides that are effective on common waterhemp as part of the weed management system (Bradley and Massey 2006).

Early season weed management is critical to optimize yields. Therefore, preemergence herbicides are effective tools to reduce weed competition. *S*-metolachlor is widely used to control common waterhemp, including herbicide-resistant biotypes; however, it frequently provides inconsistent common waterhemp control. Therefore, tank mixing metribuzin with *S*-metolachlor is done to broaden the weed spectrum and increase efficacy. Metribuzin, however, may cause soybean injury especially with improper incorporation, soybean planted shallow, or if a sensitive cultivar is used (Street et al. 1987).

Research has shown that fomesafen applied preemergence can effectively control *Amaranthus* species, including common waterhemp, common cocklebur (*Xanthium strumarium*), yellow nutsedge (*Cyperus esculentus*), prickly sida (*Sida spinosa*), and *Ipomoea* species (Lunsford et al. 1998). In addition, fomesafen applied preemergence can control protox-resistant biotypes of common waterhemp that postemergence fomesafen applications fail to control.

Tank-mixing *S*-metolachlor with fomesafen, therefore, could potentially be an option for the control of common waterhemp. The objective of this research was to determine the efficacy of *S*-metolachlor tank mixed with fomesafen on common waterhemp in soybean.

MATERIALS AND METHODS

Field experiments were conducted at the Kansas State University Research Farm at Ashland Bottoms near Manhattan, KS in 2005 and 2006 and on a producer field near Sabetha, KS in 2005. The soil at Manhattan was a Reading silt loam (fine-silty, mixed, superactive, mesic Pachic Argiudolls), with organic matter of 2.5% and 2.8% and pH of 6.0 and 6.5 in 2005 and 2006, respectively. In Sabetha, the soil was a Judson silt loam (fine-silty, mixed, superactive, mesic Cumulic Hapludolls), with organic matter of 2.8% and a pH of 7.0. Both the Manhattan and Sabetha sites were under dryland production.

'Asgrow 3302RR' soybean was planted in 76 cm row spacing at 54,600 plants ha^{-1} at Manhattan on May 24, 2005 and May 17, 2006. At Sabetha, soybean were planted at 48,500 seeds ha^{-1} in 18 cm row spacing on May 2, 2005. Soybean plots were 3 m wide and 7.6 m long at both site. Herbicides were applied with a CO_2 pressurized backpack sprayer equipped with XR8002[1] flat fan nozzle tips and calibrated to deliver 187 L ha^{-1} at a pressure of 138 kPa.

At the Manhattan site, common waterhemp seeds were sown perpendicular to the soybean rows immediately after soybean planting. Weed seeds were incorporated in the top 0.7 cm of soil surface immediately after planting. Other weed species present at the Manhattan site included velvetleaf (*Abutilon theophrasti*), large crabgrass (*Digitaria sanguinalis*), common sunflower (*Helianthus annuus*), *setaria* spp. and common lambsquarters (*Chenopodium album*). At Sabetha, natural common waterhemp population was sufficient therefore, no common waterhemp seeds were planted. Other naturally occurring weeds at Sabetha were common cocklebur, common sunflower, ivyleaf morningglory (*Ipomoea hederacea*), large crabgrass, and Palmer amaranth (*Amaranthus palmeri*).

Preemergence herbicide treatments included *S*-metolachlor + fomesafen at 0.91 + 0.22, 1.21 + 0.28, 1.52 + 0.36, and 1.82 + 0.43 kg ha^{-1} and *S*-metolachlor + metribuzin at 0.55 + 0.14 kg ha^{-1}, respectively. These preemergence treatments were applied alone or followed by a postemergence application of glyphosate at 0.88 kg ae ha^{-1}. In addition, a single postemergence treatment of glyphosate alone and a nontreated control plot were included for comparison. Glyphosate was applied when common waterhemp was 3 to 15 cm tall. Ammonium sulfate was added to all glyphosate treatments at 3.8 kg ha^{-1}. Preemergence applications were made on May 24, 2005 and May 17, 2006 at Manhattan and May 2, 2005 at Sabetha. Postemergence applications were made on June 12, 2005 and June 22, 2006 at Manhattan and June 2, 2005 at Sabetha.

Visual injury ratings were determined 2, 4, and 8 weeks after treatment (WAT) and pre-harvest on a scale of 0 to 100%, where 0 = no control or crop injury and 100% = mortality. Common waterhemp population was determined in one m^2 per plot 18 WAT. Plants were then harvested, dried at 70 C for 96 hours and weighed.

The experimental design was a randomized complete block with four replications. Data was subjected to analysis of variance and means were separated using Fisher's Protected LSD at P = 0.05.

RESULTS AND DISCUSSION

No soybean injury was observed at Manhattan in 2005 or 2006. At Sabetha *S*-metolachlor + metribuzin caused up to 10% seedling injury with maximum injury at 4 WAT (data not shown) but plants fully recovered by 7 WAT. Injury symptoms were interveinal chlorosis, cupping, and crinkling. Injury may be attributed to pH of 7.0 thus decreasing soybean tolerance and exposing the crop to injurious concentrations in soil solution (Street et al. 1987).

At Manhattan, general weed control with *S*-metolachlor + fomesafen ranged from 85 to 94% and 74 to 93% at 2 and 4 WAT in 2005, respectively (Table 1.1). By 8 WAT the two lowest rates of *S*-metolachlor + fomesafen controlled between 63 and 55% of weeds while the two highest rates controlled between 86 and 75% of weeds. General weed control with *S*-metolachlor + metribuzin at 2, 4, and 8 WAT was 85, 66, and 53%, respectively. In general, application of glyphosate after preemergence herbicide treatments increased weed control compared to preemergence herbicide treatment or glyphosate applied alone.

In 2006, general weed control with *S*-metolachlor + fomesafen 2 and 4 WAT, regardless of the rate, was greater than 93% (Table 1.1) whereas 8 WAT, weed control ranged from 70 to 90%. *S*-metolachlor + metribuzin gave greater than 95% general weed control at all rating dates. The lower weed control with *S*-metolachlor + fomesafen compared to *S*-metolachlor + metribuzin was mainly due to new flushes of velvetleaf and other large seeded broadleaves that emerged 4 WAT. General weed control 8 WAT was 100% when glyphosate was applied after preemergence herbicide treatment.

At Sabetha, general weed control with *S*-metolachlor + fomesafen ranged from 66 to 90% and 54 to 73% at 2 and 4 WAT, respectively (Table 1.2). At 8 WAT, general weed control was greater than 4 WAT and ranged from 67 to 80%. Stephenson et al. (2004) reported that weed control with fomesafen may be greater later in the growing season due to fomesafen reactivation by rainfall. General weed control with *S*-metolachlor + metribuzin was 60, 47, and 57% at 2, 4, and 8 WAT, respectively. General weed control with preemergence treatments followed by glyphosate was greater then 95%. At 4 and 8 WAT glyphosate applied alone gave 90 and 88% general weed control, respectively.

At 2 WAT, common waterhemp control at Manhattan was greater than 88% with *S*-metolachlor + fomesafen. By 4 WAT common waterhemp control dropped below 80% for the two lowest rates, but was still above 85% for the two highest rates (Table 1.3). Similarly, *S*-metolachlor + metribuzin common waterhemp control decreased from 82% at 2 WAT to 61% by 4 WAT. The decline in common waterhemp control at 4 WAT may be attributed to 3 days of heavy rainfall totaling 18 cm at 2 WAT that may have leached the herbicide in the soil and stimulated late germination of common waterhemp. *S*-metolachlor, fomesafen, and metribuzin are relatively water soluble with solubility of 488, 600000, and 1100 mg L^{-1}, respectively (Vencill 2002). Again, glyphosate application after preemergence treatments gave greater than 95% common waterhemp control. Glyphosate applied alone controlled 89% of common waterhemp.

At soybean pre-harvest, common waterhemp control with the two highest rates of *S*-metolachlor + fomesafen was greater than 78%, whereas *S*-metolachlor + metribuzin control was 37%. The differences in control between *S*-metolachlor + fomesafen and *S*-

metolachlor + metribuzin may be attributed to rapid degradation of metribuzin in the soil. The half-life of fomesafen and metribuzin are 100 and 40 days, respectively (Ahrens 1994). When glyphosate was applied following any preemergence treatment, common waterhemp control was greater than 92% whereas glyphosate applied alone controlled only 74% of common waterhemp.

Common waterhemp control with *S*-metolachlor + fomesafen at the two highest rates and *S*-metolachlor + metribuzin was greater than 98% at 2, 4 and 8 WAT at Manhattan in 2006 (Table 3). Greater herbicide activity in 2006, compared to 2005, may be attributed to adequate soil moisture after preemergence herbicide applied in 2006. Approximately 1.3 cm of rainfall was received immediately after preemergence herbicide application that may have facilitated herbicide activation into the soil. Common waterhemp control was near perfect with preemergence herbicide treatments; therefore glyphosate applications did not provide any additional control. At pre-harvest, all herbicide treatments gave greater than 90% control of common waterhemp.

At Sabetha, *S*-metolachlor + fomesafen gave greater than 95% common waterhemp control 4 and 8 WAT (Table 4). Similarly, *S*-metolachlor + metribuzin was greater than 90% at 4 and 8 WAT. Application of glyphosate after preemergence treatment slightly improved common waterhemp control compared to preemergence herbicides applied alone. Glyphosate applied alone gave 96 and 94% control at 4 and 8 WAT, respectively. Common waterhemp control at pre-harvest was 90% or greater with all herbicide treatments, except the single application of glyphosate which gave 62% control.

In 2005, *S*-metolachlor + fomesafen treatments decreased common waterhemp population 66 to 86% (Table 5) whereas, *S*-metolachlor + metribuzin decreased plant population by 7%. When glyphosate was applied following any preemergence treatment of *S*-metolachlor + fomesafen or *S*-metolachlor + metribuzin, common waterhemp population was reduced by 100 and 99%, respectively. Glyphosate applied alone reduced common waterhemp population by 53%. Common waterhemp population response to herbicide treatments in 2006 showed similar response to herbicide treatments as in 2005. In addition, at Sabetha all plots treated with a preemergence herbicides had no common waterhemp present.

In 2005, total common waterhemp biomass after treatment with the two highest rates of *S*-metolachlor + fomesafen was reduced by 80% whereas; biomass of common waterhemp treated with *S*-metolachlor + metribuzin had over 2.5 times the amount as the nontreated plot (Table 1.5). Greater common waterhemp biomass in the *S*-metolachlor + metribuzin treatments may be attributed to few large plants that survived herbicide treatments and were more competitive at lower populations. All preemergence treatments followed by glyphosate resulted in greater than 95% plant biomass reduction. Common waterhemp biomass was reduced greater than 98% with *S*-metolachlor + fomesafen or *S*-metolachlor + metribuzin, in 2006. All glyphosate applications resulted in no common waterhemp biomass. At Sabetha, common waterhemp biomass response to herbicide treatments followed the same pattern of the Manhattan site in 2006.

This research showed that *S*-metolachlor + fomesafen gave excellent early season control of common waterhemp at both Sabetha and Manhattan. The tank mix of *S*-metolachlor + fomesafen, however, was not as effective on large seeded broadleaf weeds, such as common cocklebur, common sunflower, and velvetleaf. When comparing *S*-metolachlor + fomesafen at the 1.52+0.36 kg ha^{-1} rate and *S*-metolachlor + metribuzin, the fomesafen combination gave greater weed control than the metribuzin.

SOURCES OF MATERIALS

[1]Teejet XR8002 tip. Spraying Systems Co., North Avenue, Wheaton, IL 60188.

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Table 1.1 General weed control 2, 4, and 8 weeks after treatment (WAT) with preemergence herbicides at Manhattan in 2005 and 2006.

		2005			2006		
Treatment[a]	Rate kg ha^{-1}	2 WAT	4 WAT	8 WAT	2 WAT	4 WAT	8 WAT
		% Control					
S-Metolachlor + fomesafen	0.91+0.22	94	77	63	97	93	70
S-Metolachlor + fomesafen	1.21+0.28	85	74	55	98	96	83
S-Metolachlor + fomesafen	1.52+0.36	91	93	86	99	97	90
S-Metolachlor + fomesafen	1.82+0.43	94	91	75	97	95	82
S-Metolachlor+fomesafen/ glyphosate	0.91+0.22/0.88	86	65	95	97	95	100
S-Metolachlor+fomesafen/ glyphosate	1.21+0.28/0.88	82	86	98	99	97	100
S-Metolachlor+fomesafen/ glyphosate	1.52+0.36/0.88	97	90	93	97	96	100
S-Metolachlor+fomesafen/ glyphosate	1.82+0.43/0.88	97	90	93	99	96	100
S-Metolachlor+metribuzin	0.55+0.14	85	66	53	98	98	96
S-Metolachlor+metribuzin/ glyphosate	0.55+0.14/0.88	81	74	92	98	96	100
Glyphosate	0.88	NA	NA	84	NA	NA	100
LSD (0.05)		15	13	16	NS	NS	14

[a]All glyphosate treatments were applied postemergence 4 WAT and included ammonium sulfate at 3.8 kg ha^{-1}.

Table 1.2. General weed control 2, 4, and 8 weeks after treatment (WAT) with preemergence herbicides at Sabetha in 2005.

Treatment[a]	Rate	2 WAT	4 WAT	8 WAT
	kg ha^{-1}		% Control	
S-Metolachlor + fomesafen	0.91+0.22	68	54	70
S-Metolachlor + fomesafen	1.21+0.28	66	65	67
S-Metolachlor + fomesafen	1.52+0.36	81	67	75
S-Metolachlor + fomesafen	1.82+0.43	90	73	80
S-Metolachlor+fomesafen/ glyphosate	0.91+0.22/0.88	61	98	98
S-Metolachlor+fomesafen/ glyphosate	1.21+0.28/0.88	78	93	98
S-Metolachlor+fomesafen/ glyphosate	1.52+0.36/0.88	84	98	98
S-Metolachlor+fomesafen/ glyphosate	1.82+0.43/0.88	90	95	98
S-Metolachlor+metribuzin	0.55+0.14	60	47	57
S-Metolachlor+metribuzin/ glyphosate	0.55+0.14/0.88	53	95	98
Glyphosate	0.88	NA	90	88
LSD (0.05)		19	12	12

[a]All glyphosate treatments were applied postemergence 3 WAT and included ammonium sulfate at 3.8 kg ha^{-1}.

Table 1.3. Common waterhemp control 2, 4, and 8 weeks after treatment (WAT) with preemergence herbicides and at pre-harvest (PH) at Manhattan, KS in 2005 and 2006.

		2005				2006			
Treatment[a]	Rate	2 WAT	4 WAT	8 WAT	PH	2 WAT	4 WAT	8 WAT	PH
	kg ha^{-1}	% Control							
S-Metolachlor + fomesafen	0.91+0.22	97	77	70	57	100	98	85	93
S-Metolachlor + fomesafen	1.21+0.28	88	67	60	44	100	98	100	100
S-Metolachlor + fomesafen	1.52+0.36	90	88	87	78	100	99	100	99
S-Metolachlor + fomesafen	1.82+0.43	94	95	76	82	100	100	99	100
S-Metolachlor+fomesafen/ glyphosate	0.91+0.22/0.88	88	65	98	96	100	100	100	100
S-Metolachlor+fomesafen/ glyphosate	1.21+0.28/0.88	84	89	100	96	100	100	98	100
S-Metolachlor+fomesafen/ glyphosate	1.52+0.36/0.88	98	94	95	99	100	100	100	100
S-Metolachlor+fomesafen/ glyphosate	1.82+0.43/0.88	99	92	100	98	100	100	100	100
S-Metolachlor+metribuzin	0.55+0.14	82	61	59	37	98	100	100	100
S-Metolachlor+metribuzin/ glyphosate	0.55+0.14/0.88	93	66	97	92	99	98	100	100
Glyphosate	0.88	NA	NA	89	74	NA	NA	100	100
LSD (0.05)		NS	15	15	19	1	NS	5	3

[a]All glyphosate treatments were applied postemergence 4 WAT and included ammonium sulfate at 3.8 kg ha^{-1}.

Table 1.4. Common waterhemp control 2, 4, and 8 weeks after treatment (WAT) with preemergence herbicides and at pre-harvest (PH) at Sabeth in 2005.

Treatment[a]	Rate	2WAT	4 WAT	8 WAT	PH
	kg ha^{-1}	% Control			
S-Metolachlor + fomesafen	0.91+0.22	100	97	95	96
S-Metolachlor + fomesafen	1.21+0.28	100	96	98	99
S-Metolachlor + fomesafen	1.52+0.36	100	98	98	93
S-Metolachlor + fomesafen	1.82+0.43	100	100	100	96
S-Metolachlor+fomesafen/glyphosate	0.91+0.22/0.88	100	100	100	99
S-Metolachlor+fomesafen/glyphosate	1.21+0.28/0.88	100	100	100	93
S-Metolachlor+fomesafen/glyphosate	1.52+0.36/0.88	100	98	100	100
S-Metolachlor+fomesafen/glyphosate	1.82+0.43/0.88	100	100	100	90
S-Metolachlor+metribuzin	0.55+0.14	100	91	91	90
S-Metolachlor+metribuzin/glyphosate	0.55+0.14/0.88	98	99	100	100
Glyphosate	0.88	NA	96	94	62
LSD (0.05)		1	3	5	9

[a]All glyphosate treatments were applied postemergence 3 WAT and included ammonium sulfate at 3.8 kg ha^{-1}.

Table 1.5. Common waterhemp population and plant biomass as affected by herbicide treatments applied on soybean at Manhattan in 2005 and 2006 and Sabetha in 2005.

		Population			Biomass		
		Manhattan		Sabetha	Manhattan		Sabetha
Treatment[a]	Rate	2005	2006	2005	2005	2006	2005
	kg ha^{-1}	Plant m^{-2}			g dry weight m^{-2}		
Control		120	64	16	160	176	32
S-Metolachlor + fomesafen	0.91+0.22	24	2	0	64	160	0
S-Metolachlor + fomesafen	1.21+0.28	40	0	0	256	0	0
S-Metolachlor + fomesafen	1.52+0.36	16	0	0	32	0	0
S-Metolachlor + fomesafen	1.82+0.43	16	0	0	32	0	0
S-Metolachlor+fomesafen/glyphosate	0.91+0.22/0.88	0	0	0	0	0	0
S-Metolachlor+fomesafen/glyphosate	1.21+0.28/0.88	0	0	0	0	0	0
S-Metolachlor+fomesafen/glyphosate	1.52+0.36/0.88	0	0	0	0	0	0
S-Metolachlor+fomesafen/glyphosate	1.82+0.43/0.88	0	0	0	0	0	0
S-Metolachlor+metribuzin	0.55+0.14	112	0	0	424	0	0
S-Metolachlor+metribuzin/glyphosate	0.55+0.14/0.88	2	0	0	2	0	0
Glyphosate	0.88	56	0	16	8	0	32
LSD (0.05)		31	18	6	40	61	12

[a]All glyphosate treatments were applied postemergence and included ammonium sulfate at 3.8 kg ha^{-1}.

CHAPTER 2 - Protox-resistant Common Waterhemp Competitiveness

ABSTRACT

Research was conducted to determine the competitiveness and fitness of a protox-resistant common waterhemp (*Amaranthus rudis*) biotype. Protox-resistant and protox-susceptible biotypes were grown under noncompetitive and competitive arrangements in the greenhouse. In the noncompetitive study a single plant of each biotype was planted separately in 15-cm-diam pots. Photosynthesis, leaf area, and plant biomass were measured 10, 20, 30, and 40 day after transplanting (DATP). In general, photosynthesis rate and plant biomass was similar between biotypes. However, the protox-resistant biotype had higher leaf area than the susceptible biotype at 20, 30, and 40 DATP. Under competitive conditions, a replacement series study, photosynthesis, leaf area, plant height, and plant biomass were measured 7, 14, 21, and 28 DATP. In general protox-resistant and –susceptible common waterhemp values were similar 28 DATP. Relative crowding coefficient values 28 DATP were 0.86, 0.89, 1.09, and 1.13 for photosynthesis, leaf area, plant height, and plant biomass, respectively. This Suggests protox-resistant and –susceptible common waterhemp were equally competitive.

Key words: Herbicide resistance, Protox-inhibitor herbicides, protox resistance, competitiveness

INTRODUCTION

Common waterhemp (*Amaranthus rudis*) is an annual C_4 weed species that has rapid growth characteristics, and extended seedling emergence pattern (Horak and Loughin 2000; Hartzler et al. 1999). In addition, common waterhemp produces up to 2 million seed per plant, and can attain heights of 2 to 3 meters, and can have detrimental effects on crop yield (Battles et al. 1998;Bensch et al. 2003). Soybean (*Glycine max*) yield reduced by 19 and 34% with common waterhemp competition for six and eight weeks after soybean unifoliate expansion (Hager 2002). Grain sorghum (*Sorghum bicolor*) yield was reduced 45% when three common waterhemp plants were allowed to compete with grain sorghum for 10 weeks (Feltner et al. 1969). In addition, Steckel and Sprague (2004) reported that corn (*Zea mays*) yield loss was 74% when 270 plants m^{-2} were allowed to compete beyond the V10 stage.

Common waterhemp control in soybean has been achieved with many herbicides including chloroacetamides, glyphosate, metribuzin, dinitroanilines, ALS-inhibiting herbicides, and protoporphyrinogen oxidase-inhibiting herbicides (Mayo et al. 1995; Regehr et al. 2003; Sweat et al. 1998; Shoup and Al-Khatib 2004). However, Heap (2007) reports that common waterhemp with resistance to four modes of action have been identified in the United States including ALS-inhibitors, glycines, photosystem II-inhibitors, and protox-inhibiting herbicides. The first case of common waterhemp with resistance to protox-inhibiting herbicides was discovered in 2000 in northeast Kansas (Shoup et al. 2003).

Protoporphyrinogen oxidase (protox)-inhibiting herbicides contain eight different chemistries of protox-inhibiting herbicides including diphenyl ethers, oxadiazoles, N-phenylphthalimides, phenylpyrazoles, oxazolidinediones, pryimidindiones, triazolinones, and thiadiazoles. However, herbicides belonging to three chemistries are commercially available to producers in the United States including diphenyl ether, triazolinones, and N-phenyplphthalimide. Herbicides belonging to these three chemistries, including fomesafen, lactofen, aciflurofen, flumioxazen, and sulfentrazone, can effectively control common waterhemp (Regehr et al. 2003; Sweat et al. 1998; Falk 2005).

Oxidation of protoporphyrinogen IX (protogen) to protoporphyrin IX (proto) is the last step in the tetrapyrrole–synthesis pathway before it branches to chlorophyll or heme synthesis (Beale and Weinstein 1990). When protox is inhibited, protogen will leak out of the plastid and is rapidly oxidized to proto IX by herbicide-resistant peroxidases that are nonspecific and bound to the plasma membrane (Jacobs and Jacobs 1993). Highly reactive singlet oxygen generated by light activation of proto IX, a photodynamic tetrapyrrole, causes rapid peroxidation of the membrane, resulting in serious cell membrane damage (Becerril and Duke, 1989). Single alteration in the plastidic or mitochondrial protox enzyme is suggested to induce protox-resistance (Patzoldt et al. 2003).

Change in plant fitness is one potential result of herbicide resistant gene(s). A major concern associated with herbicide resistant genes is the risk of fitness related genes, resulting in a more invasive and noxious weeds (Ellstrand 1999). However, herbicide resistant genes also may result in growth reduction (Radosevich 1997).

Fitness measures that describe the potential success of a genotype should be based on survival, competition, and reproduction (Holt 1990). The most fit plants produce the greatest number of offspring and contribute greater proportion of its genes to total gene pool of the population (Radosevich 1997).

Sibony and Rubin (2002) showed that ALS-resistant and -susceptible biotypes of *Amaranthus retroflexus* expressed similar ecological fitness. Further studies conducted show that ALS-resistant genes in downy brome, prostrate pigweed (*Amaranthus blitoides)*, common cocklebur (*Xanthium strumarium*) common sunflower (*Helianthus annuus*), and prairie sunflower (*Helianthus petiolaris*)did not result in growth penalty associated with this trait (Park et al. 2004; Massinga et al. 2005; Sibony and Rubin 2002; and Crooks et al. 2005). These results may indicate that in the absence of ALS inhibitors the resistant biotype will remain at a similar frequency in a population of resistant and susceptible plants. However, triazine-resistant weed biotypes almost always are less vigorous than susceptible biotypes of the same species (Vaughn 2003). Similar finding have been reported in *Datura stramonium*, *Amaranthu*s spp., *Brassicus napus*, *Chenopodium album*, *Senecio vulgaris*, and *Poa annua* (McCloskey 1990; Warwick 1991). The results showed that the triazine-resistant gene had a fitness cost to the plant.

The objective of this study was to determine the competitiveness of the protox-resistant common waterhemp in a non-competitive and competitive setting, and if the protox-resistant gene has a fitness cost to the plant.

MATERIALS AND METHODS

Plant Materials. Common waterhemp seeds were collected from a known protox-resistant population in a soybean field near Sabetha, KS (Shoup 2003). Susceptible common waterhemp seeds were collected from Emporia, KS where no past protox-inhibiting herbicides had been used in the last 20 years.

The protox-resistant and –susceptible biotypes of common waterhemp were evaluated for resistance to triazine and ALS-inhibiting herbicides to ensure that the two biotypes used in this study were susceptible to these herbicides. The protox-resistant and -susceptible seedlings at a height of 15-20 cm were cut at the top of the fifth node and the cut plant parts were propagated. The propagated plants were tagged with a corresponding number back to the original parent plant for identification purposes. Once the propagated plants had regained growth vigor approximately three weeks after propagation, plants were treated with the recommended use rate of atrazine or imazethapyr. The recommended use rates were 70.6 g a.i.ha^{-1} and 2.24 kg a.i.ha^{-1} for imazethapyr and atrazine, respectively. Herbicide application was made with a bench-type sprayer[2] calibrated to deliver 187 L ha^{-1} at 138 kPa. The parent of the propagated plants from both biotypes that were killed by atrazine and imazethapyr were kept. The corresponding parent plant of the atrazine and imazethapyr susceptible plants were then grown for seed and used in the study. This screening resulted in two biotypes of common waterhemp, one that had resistance only to protox-inhibiting herbicides but were susceptible to atrazine and imazethapyr, and a second biotype that was susceptible to all three classes of herbicides.

Protox-resistant and –susceptible common waterhemp were grown in 50 cm x 35 x 10 cm container. At the 3rd node growth stage, plants were transplanted into 15-cm-diam pots filled with 500 g soil, and 34 x 28 x 12 cm plastic containers containing 9 kg of soil for noncompetitive and competitive conditions, respectively. Soil was a 1:1 (v/v) mixture of sand and Morill loam (mesic Typic Argiudolls) with pH 6.8 and 1.9% organic matter. Plants were watered as needed and fertilized weekly with commercial fertilitzer[1] containing 300 mg L^{-1} nitrogen, 250 mg L^{-1} phosphorus, and 220 mg L^{-1} potassium. Greenhouse conditions were 26/20 ^{o}C day/night temperature and 14/10 h day/night photoperiod. Supplemental light was at 80 μmol m^{-2} s^{-1} photosynthetic photon flux photoperiod.

Non-competitive Study. Photosynthesis, leaf area, and stem, root, and leaf dry weights were measured 10, 20, 30, and 40 days after transplanting (DATP). Common waterhemp were approximately 15, 40, 60 and 80 cm at 10, 20, 30, and 40 DATP, respectively. Photosynthesis rate was measured on the third fully expanded leaf from the top using a Li-Cor 6400 portable photosynthesis system[3]. Leaf area was measured with Li-Cor 3100 Area Meter[3]. Plant parts were dried at 60 ^{o}C for 72 hours and weighed.

Competitive Study. Common waterhemp seedlings were transplanted into containers as described above at the following ratios of protox-resistant:protox-susceptible: 6:0, 5:1, 4:2, 3:3, 2:4, 1:5, 0:6, respectively (Marshall et al 2001). Plants were spaced 8 to 9 cm apart. Photosynthesis, leaf area, plant height, and stem and leaf dry weights were measured 7, 14, 21, and 28 DATP as described above. Common waterhemp were similar in size to the noncompetitive study at time of measurement.

Relative crowding coefficient (RCC) is used to determine the competitive ability of a plant to obtain limited resources when grown in a community setting as when compared to its ability to utilize those resources when grown in a monoculture setting. According to this definition, a RCC value that is greater than 1.0 signifies a competitive advantage for the protox-resistant biotype when compared to the protox-susceptible biotype, whereas, when the value is less than 1.0 the protox-susceptible biotype is more competitive than the protox-resistant biotype. A RCC value of 1.0 indicates that there is no competitive advantage or disadvantage between biotypes. The RCC values were calculated at each harvest date according to Novak's equation (1993):

$$\frac{\left[\left(\frac{Y_{(5:1\ \mathrm{Resistant})}}{Y_{(5:1\ \mathrm{Susceptible})}} + \frac{Y_{(4:2\ \mathrm{Resistant})}}{Y_{(4:2\ \mathrm{Susceptible})}} + \frac{Y_{(3:3\ \mathrm{Resistant})}}{Y_{(3:3\ \mathrm{Susceptible})}} + \frac{Y_{(2:4\ \mathrm{Resistant})}}{Y_{(2:4\ \mathrm{Susceptible})}} + \frac{Y_{(1:5\ \mathrm{Resistant})}}{Y_{(1:5\ \mathrm{Susceptible})}}\right) / N\right]}{\left(\frac{Y_{(6:0\ \mathrm{Resistant})}}{Y_{(6:0\ \mathrm{Susceptible})}}\right)}$$

where Y is average photosynthesis, height, leaf area, or dry biomass.

Both experiments were conducted as randomized complete bock designs and experiments were repeated with four and eight replications for non-competitive and competitive studies, respectively. No run by treatment interactions occurred were observed data was averaged across runs. Plant photosynthesis, height, leaf area, and dry weight data were tested using ANOVA, and means were separated using LSD at the P = 0.05 level.

RESULTS AND DISCUSSION

Non-competitive Study. Morphologically and phenotypically the protox-resistant and –susceptible biotypes were similar with no distinguishing characteristics between the two biotypes. Similarly, Marshall (2001) and Massinga et al. (2005) reported no morphological differences between imazethapyr-resistant and -susceptible sunflowers (*Helianthus annuus*) and prairie sunflower (*Helianthus petiolaris*).

In general photosynthesis rates for protox-resistant and –susceptible common waterhemp biotypes were similar at all harvest dates (Figure 2.1). However, the rate of photosynthesis in the resistant common waterhemp was higher than susceptible plants 20 DATP, but these differences disappeared by 30 DATP. The leaf area of protox-resistant common waterhemp was greater than susceptible at 30 DATP, but the difference had decreased by 40 DATP (Figure 2.2). In contrast, total plant biomass of protox-resistant and –susceptible common waterhemp biotypes were similar throughout the study (Figure 2.3). Although, the protox-resistant biotype had greater leaf area than the protox-susceptible biotype, no difference was observed in total plant biomass due to increased branching and greater stem weight for the susceptible biotype (data not shown). In both biotypes, the lack of differences in photosynthesis, leaf area and total plant biomass at the end of the study indicate that the two biotypes when grown individually exhibit similar growth characteristics. Numerous studies showed similar results in ALS resistance with no competitive advantage or disadvantage being observed in ALS-resistant common sunflower, prairie sunflower, common cocklebur, and downy brome (Massinga et al 2005; Crooks et al. 2005; Park et al. 2004).

Competitive Study. Replacement series diagrams illustrate competitive effects between tested plants (Massinga et al. 2005). Equal competition between biotypes would be represented by straight lines across the mixture proportions (Figure 2.4), with the intersection at the 50% of the mixture ratio, whereas, curved lines shifting the intersection point away from the 3:3 mixture ratio indicate that competitive differences exist (Anderson et al. 1996).

Plants were harvested earlier and with shorter intervals in the competitive study, compared to the noncompetitive study, to determine if any differences existed in early growth stages. Photosynthesis rate was similar in both protox-resistant and -susceptible common waterhemp where each biotype was grown in pure stand, throughout the study (Figure 2.4). Photosynthesis rate 7 DATP shifted left of the 3:3 ratio indicating a competitive advantage for the resistant biotype when compared to the susceptible biotype. However, 28 DATP photosynthesis of the resistant and susceptible plants were similar and the intersection line was at the 3:3 ratio. When grown in pure stand, leaf area for both biotypes was similar (Figure 2.5). Replacement series diagrams for leaf area 7 DATP show that the lines were intersect at the 2:4 ratio suggesting that resistant plants are more competitive than susceptible plants. This competitive advantage is no longer evident 28 DATP as the intersection line was at the 3:3 intersection. The susceptible biotype had more branches and higher stem weight than the resistant biotype, while the resistant biotype had higher leaf weight (data not shown). When grown in pure stand or in mixture combination, no differences in plant height were observed between the two biotypes at 7 or 28 DATP (Figure 2.6). In general, when comparing plant biomass of protox-resistant and –susceptible biotypes under pure stand, no differences were observed. However, 7 DATP the intersection point for plant biomass was at the 2:4 ratio, indicating that protox-resistant biotype was more competitive. At 28 DATP, however; the intersection point for the two biotypes is shifted to the 3:3 ratio indicating equal competition between protox-resistant and –susceptible biotypes of common waterhemp.

The RCC values for 7 DATP are 1.39, 1.24, 1.15, and 1.29 for photosynthesis, leaf area, plant height, and plant biomass respectively (Table 2.1). These values indicate that some competitive advantage for the protox-resistant biotype is evident 7 DATP. However, 14 and 21 DATP the values start decreasing towards one. In addition, 28 DATP no competitiveness was observed with RCC values of 0.86, 0.89, 1.09, and 1.13 for photosynthesis, leaf area, plant height, and plant biomass, respectively, indicating equal competitiveness between the protox-resistant and –susceptible biotypes of common waterhemp.

Patzoldt et al. (2006) reported that protox resistance in the active site of a protox enzyme was due to glycine deletion in both the chloroplast and mitochondria. Protox resistance is an incomplete dominant trait conferred by a single, nuclear gene. In this study, resistance to protox-inhibiting herbicides in common waterhemp did not reduce competitiveness when compared to a protox-susceptible biotype. This suggests that the amino acid alterations did not change the ability of common waterhemp to grow normally under noncompetitive and competitive conditions.

Early differences in photosynthesis and growth may be attributed to quality and size of seed of the protox-susceptible biotype.

Since no competitive differences exist in the frequency of protox-resistant plants will likely remain constant if protox-inhibiting herbicides are no longer used. Frequency of protox resistance will be dependent on environmental conditions, seed dispersal, and rate of gene flow from resistant plants to susceptible plants through outcrossing. Integrated weed management tactics utilizing tillage, crop rotation, and rotation of herbicides with different modes of action will decrease selection pressure for development of resistance (Falk et al. 2005).

SOURCES OF MATERIALS

[1]Miracle-Gro soluble fertilizer, Scotts Miracle-Gro Products Inc., Consumer Products Division, 1411 Socttslawn Road, Marysville, OH 43041

[2]Research Track Sprayer SB-8. Devries Manufacturing, RR 1, Box 184, Hollandale, MN 56045.

[3]LI-COR Inc., 4421 Superior Street, Lincoln, NE 68504.

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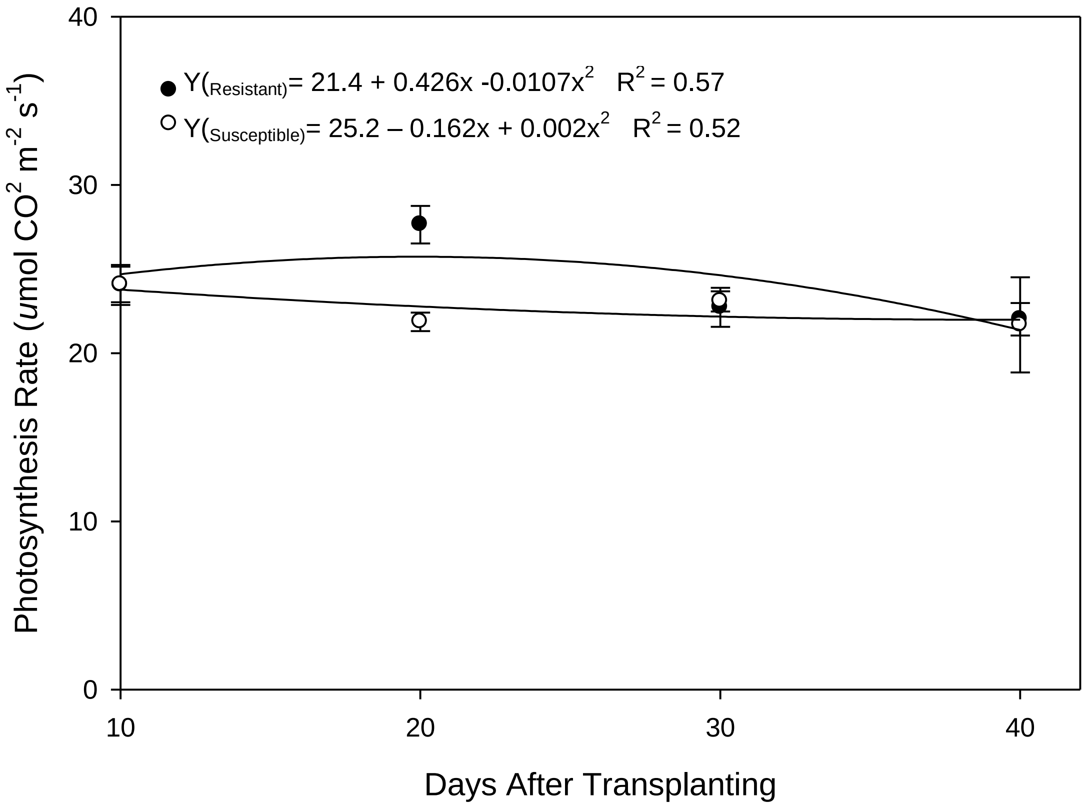

Figure 2.1. Photosynthesis of protox-resistant and -susceptible common waterhemp grown under noncompetitive conditions. Bars indicate ± standard error.

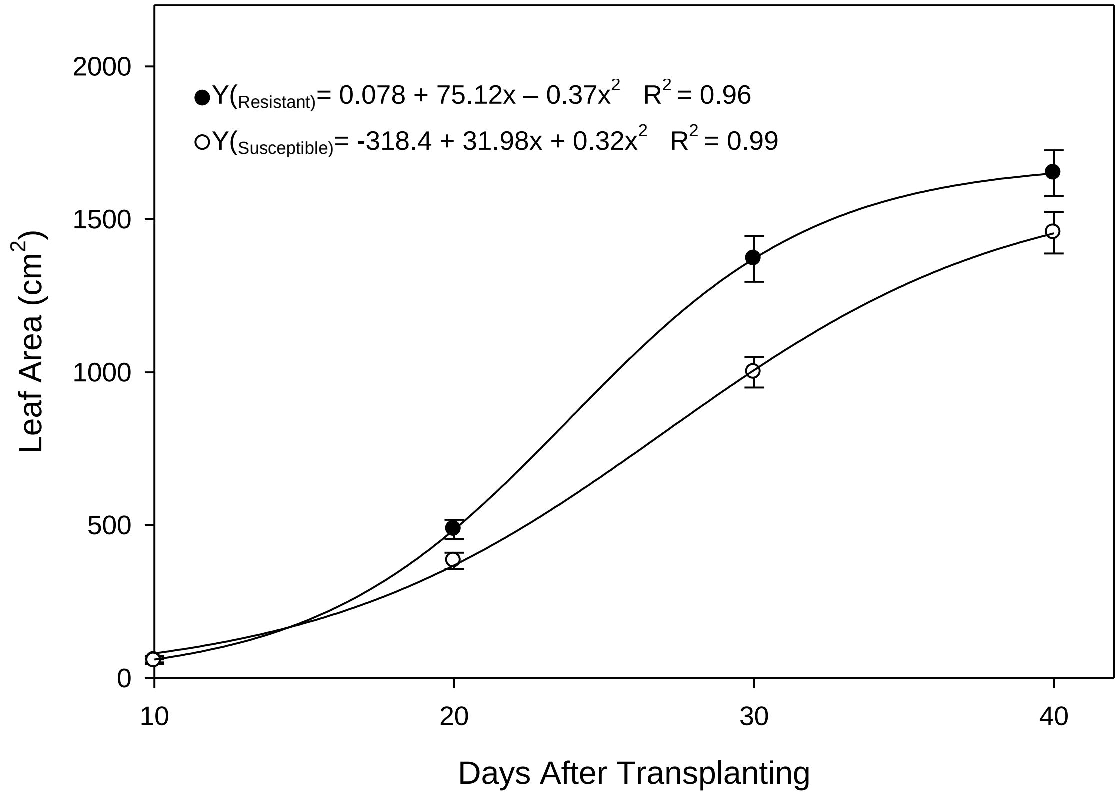

Figure 2.2. Leaf area of protox-resistant and -susceptible common waterhemp grown under noncompetitive conditions. Bars indicate ± standard error.

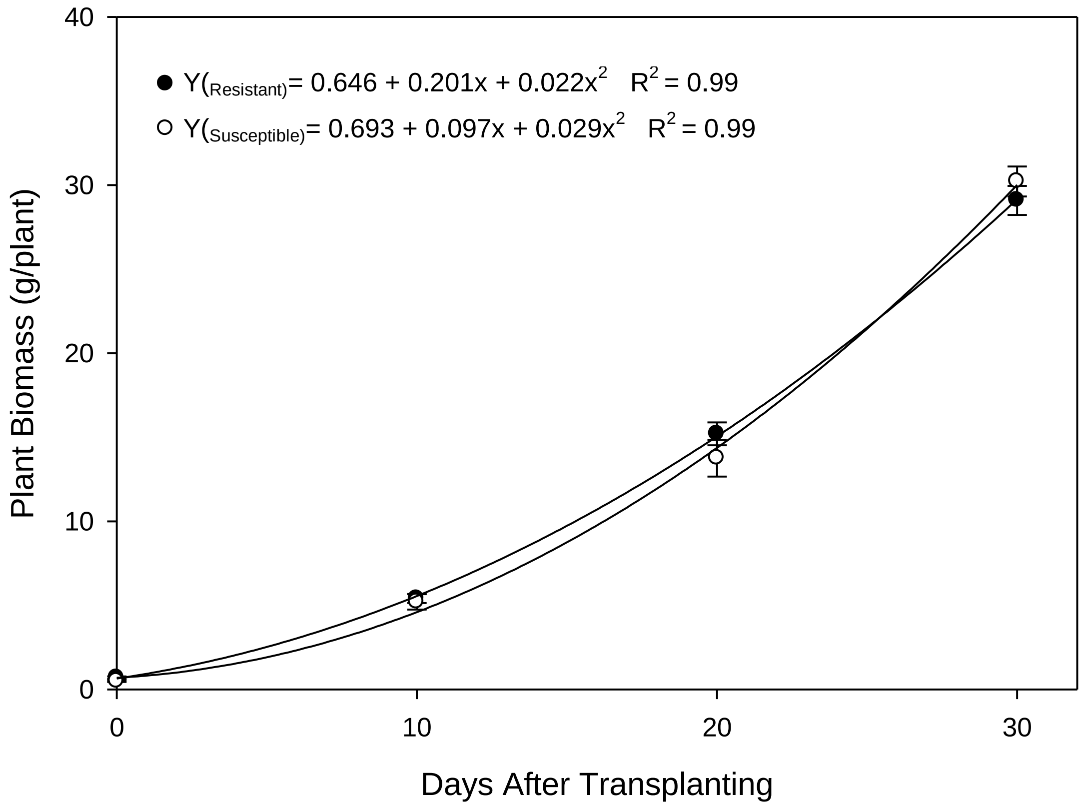

Figure 2.3. Total plant biomass of protox-resistant and -susceptible common waterhemp grown under noncompetitive conditions. Bars indicate ± standard error.

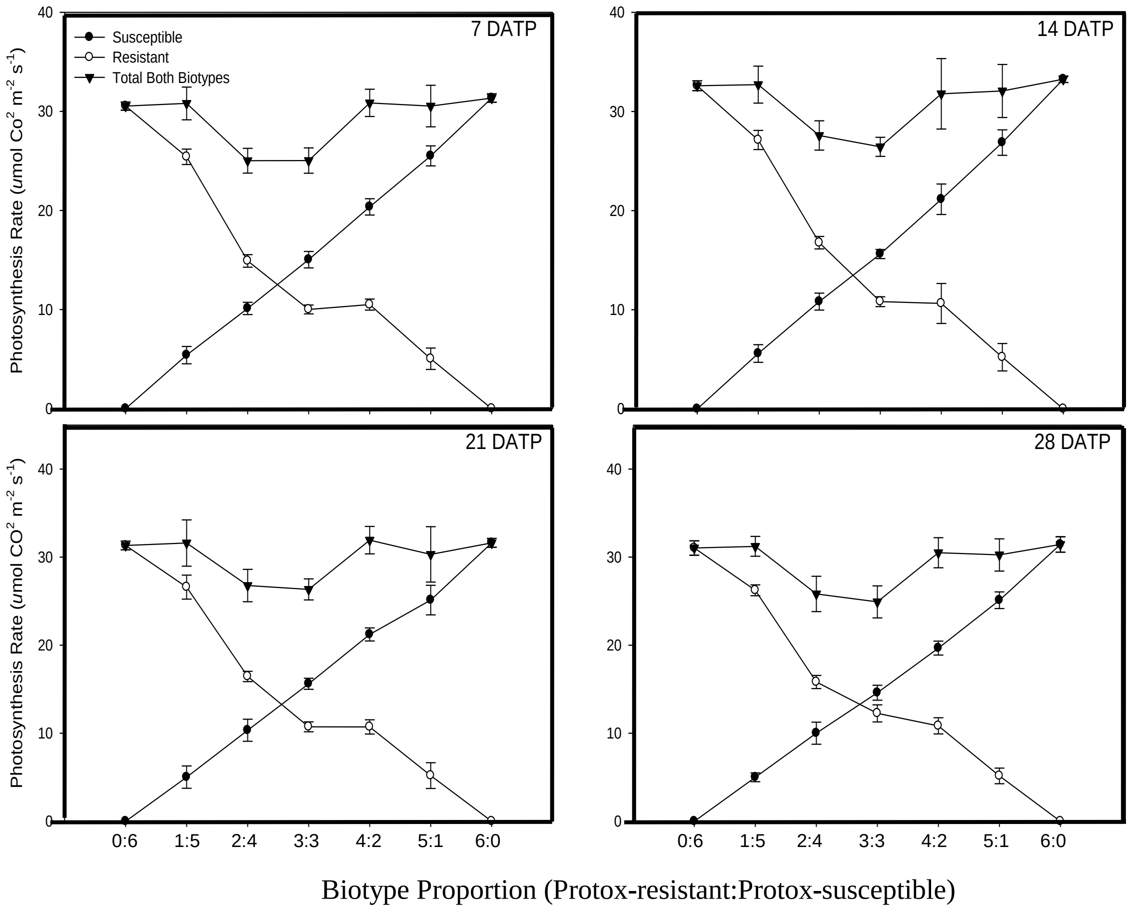

Figure 2.4. Replacement series diagrams for photosynthesis of protox-resistant and -susceptible common waterhemp at different protox-resistant and -susceptible proportions harvested 7, 14, 21, and 28 days after transplanting (DATP). Bars indicate ± standard error.

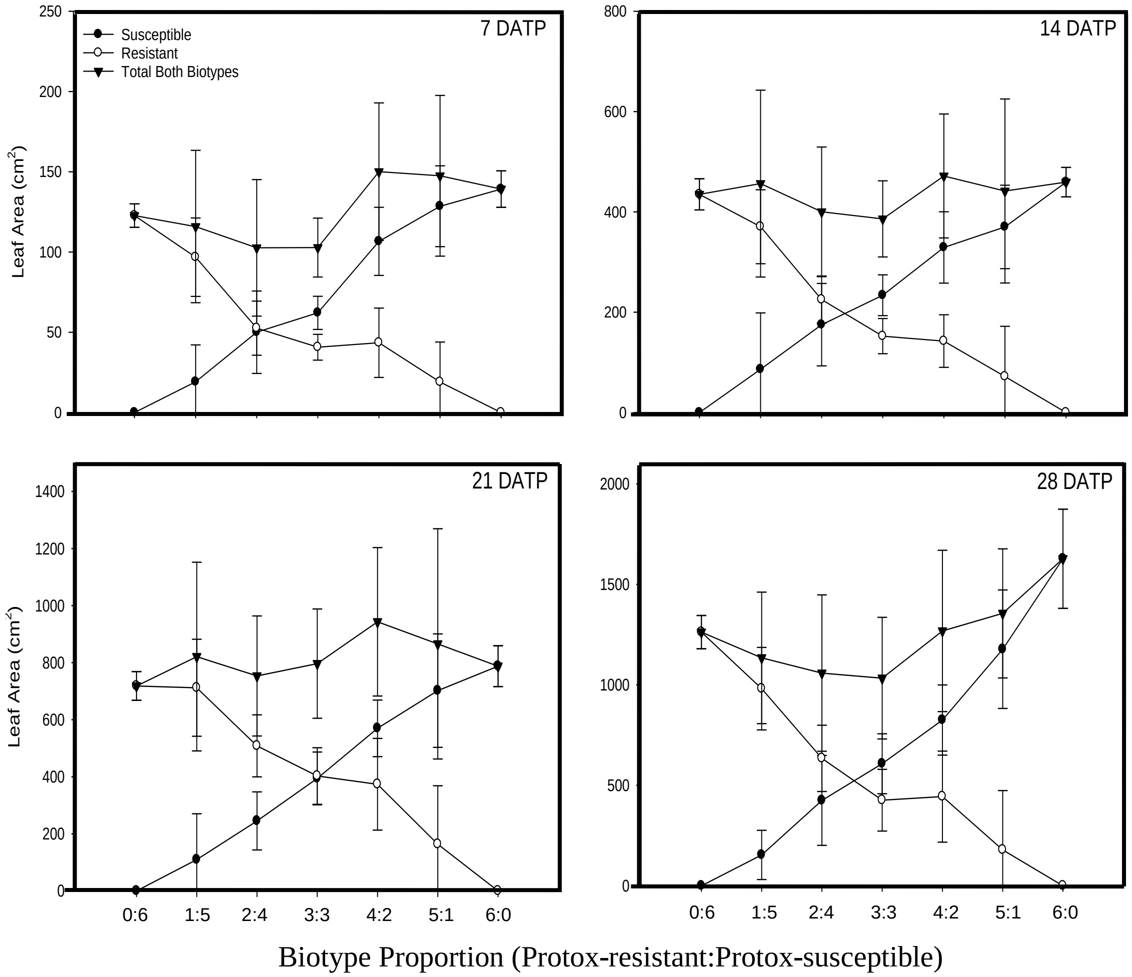

Figure 2.5. Replacement series diagrams for leaf area of protox-resistant and -susceptible common waterhemp at different protox-resistant and -susceptible proportions harvested 7, 14, 21, and 28 days after transplanting (DATP). Bars indicate ± standard error.

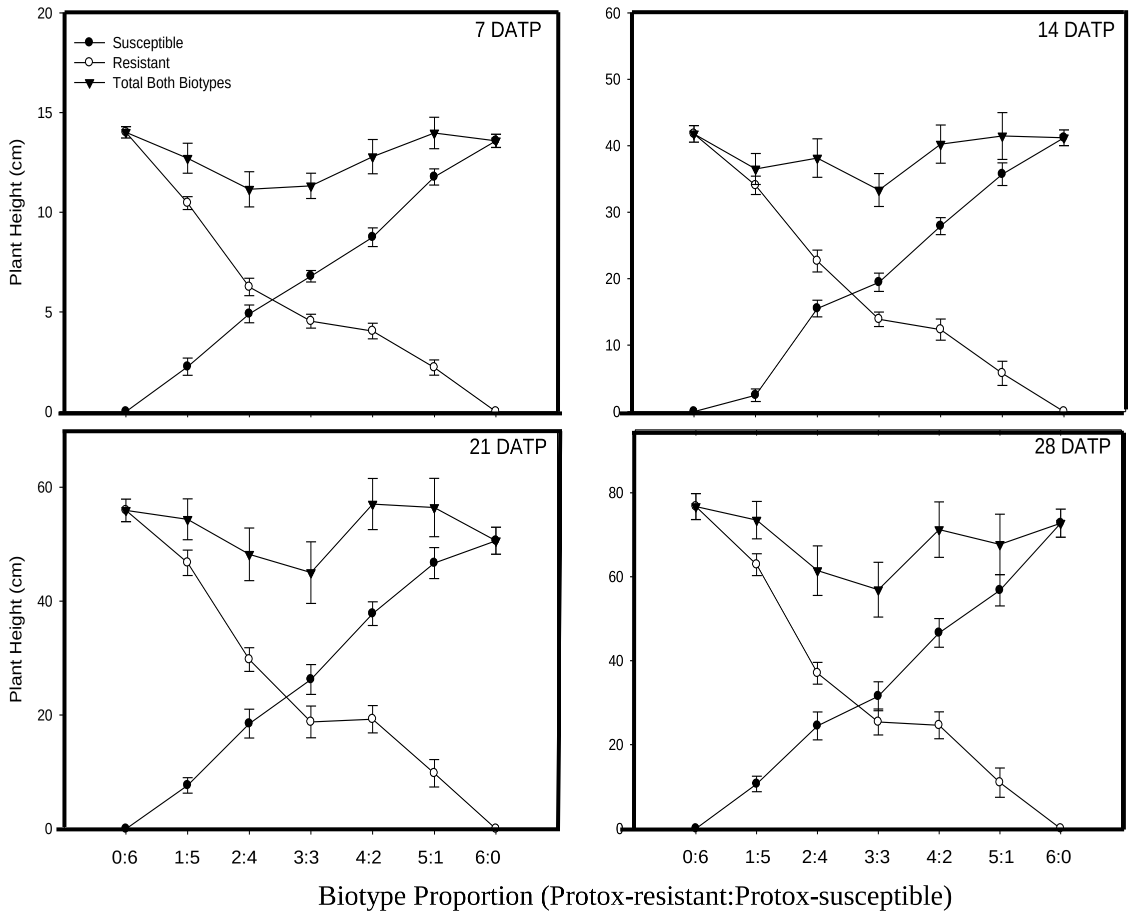

Figure 2.6. Replacement series diagrams for plant height of protox-resistant and -susceptible common waterhemp at different protox-resistant and -susceptible proportions harvested 7, 14, 21, and 28 days after transplanting (DATP). Bars indicate ± standard error.

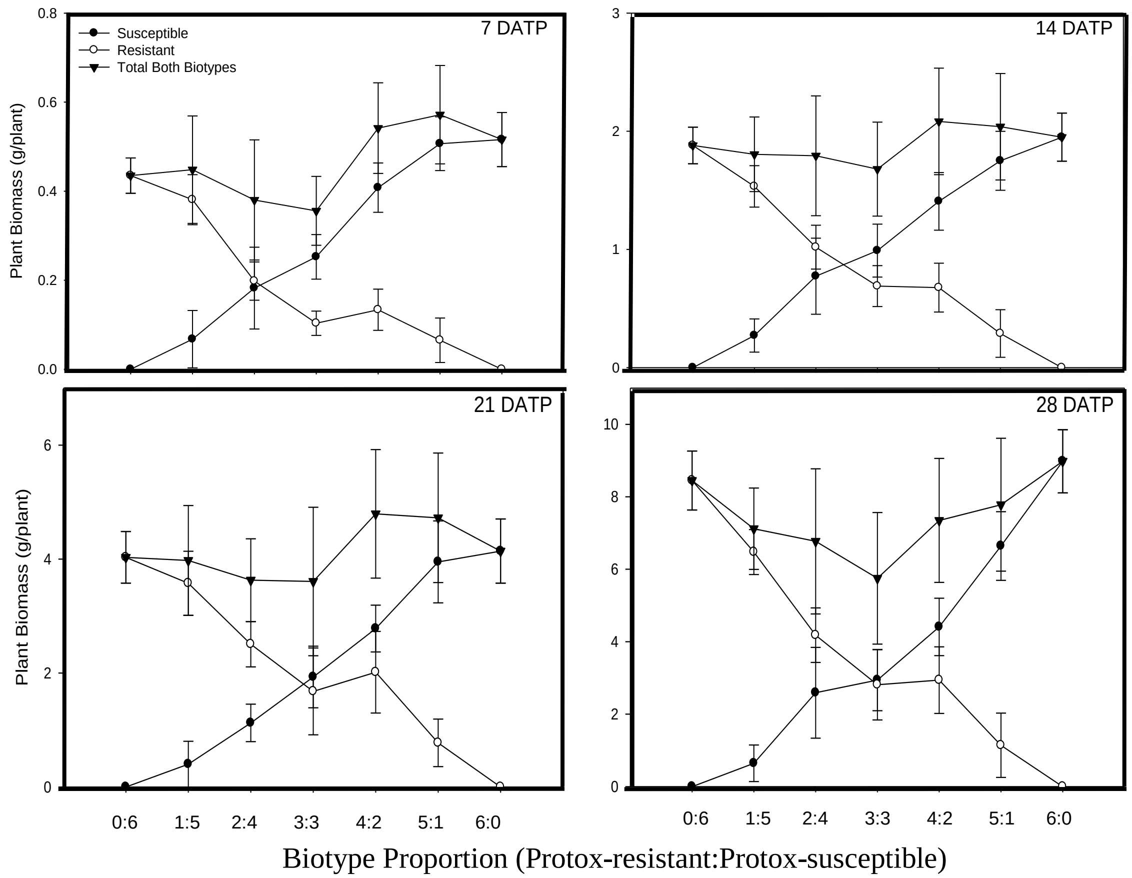

Figure 2.7. Replacement series diagrams for plant biomass of protox-resistant and -susceptible common waterhemp at different protox-resistant and -susceptible proportions harvested 7, 14, 21, and 28 days after transplanting (DATP). Bars indicate ± standard error.

Table 2.1. Relative crowding coefficient values for photosynthesis, leaf area, plant height, and plant biomass 7, 14, 21, and 28 days after transplanting.

	Relative Crowding Coefficient			
Days after transplanting	Photosynthesis	Leaf area	Height	Plant biomass
7	1.39	1.24	1.15	1.29
14	1.17	1.22	1.13	1.09
21	0.86	0.80	1.11	1.20
28	0.86	0.89	1.09	1.13